EFFECT OF NITROGEN NUTRITION ON GROWTH AND SPORULATION OF SOME PATHOGENIC FUNGI

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Nitrogen is essential for the growth of fungi which are highly specific in their food requirements. On the basis of their nitrogen requirements Robbins (1937) classified the fungi into four groups. First group can use nitrate, ammonium, organic and atmospheric nitrogen; second one needs nitrate, ammonium and organic nitrogen; the third one can utilise ammonium and organic nitrogen while the members of the fourth group depend on organic nitrogen only. Ajello (1948) reported that *Polychytrium aggregatum* gave best growth on peptone but it was unable to grow on ammonium sulphate and urea. Wolf and Shoup (1943) working on *Allomyces arbuscula*, *A. javanicus*, *A. moniliformis* and *A. cystogenus* found that they were unable to use glycine and tyrosine. Leonian and Lilly (1940) reported that 2% glycine, alanine, aspartic acid and asparagine induced zygospore formation of *Phycomyces blakesleeanus* but addition of ammonium nitrate either alone or in combination with any of the above substances completely suppressed their development. These results indicate that the growth and sporulation of fungi varied considerably on different nitrogen compounds. It was, therefore, necessary to study the nitrogen requirements of different fungi separately.

Material and Methods

The investigations were carried out with three different fungi. *Glœosporium musarum* Ck. and Mass. was isolated from bananas, *Glœosporium papayæ* P. Henn. and *Colletotrichum papayæ* P. Henn. from papayas. They were purified and then maintained on modified czapek's medium containing $NaNO_3$, 2·0 gm.; K_2HPO_4, 1·0 gm.; $MgSO_4$, 0·5 gm.; sucrose, 15·0 gm.; $FeSO_4$, 0·01 gm., and double distilled water 1000 c.c. For the study of nitrogen requirements of the fungi, sodium nitrate of the basal medium was substituted with equivalent amount of organic or inorganic nitrogen compounds containing 329 mg. of nitrogen present in czapek's medium. The amount of peptone added was equal to the weight of sodium

TABLE I

Showing average dry weights and sporulation of Glœosporium musarum, Glœosporium papayæ *and* Colletotrichum papayæ *on different nitrogen compounds added to the basal medium at the rate of* 329 *milligrams of nitrogen per litre*

S. No.	Nitrogen Compound	*G. musarum*		*G. papayæ*		*C. papayæ*		
		Average dry wt.	Sporulation	Average dry wt.	Sporulation	Average dry wt.	Sporulation	Setæ
1.	Peptone	237·2	good	205·4	good	201·4	good	absent
2.	Magnesium nitrate	218·3	do.	202·3	do.	153·0	do.	do.
3.	Glycine	216·1	fair	190·2	absent	130·9	fair	do.
4.	Ammonium acetate ..	215·8	V. fair	190·2	do.	181·8	do.	fair
5.	Urea	212·3	good	195·1	fair	141·4	good	poor
6.	Calcium nitrate	210·9	do.	160·5	good	156·5	do.	absent
7.	Glutamic acid	210·9	fair	181·2	absent	124·6	fair	fair
8.	*d*-Alanine	207·6	good	181·0	V. fair	146·2	good	do.
9.	Acetamide	201·9	do.	175·5	do.	191·6	do.	do.
10.	*l*-Asparagine	197·0	do.	184·8	fair	130·5	do.	do.
11.	Potassium nitrate	196·0	do.	169·6	good	149·3	do.	do.
12.	Sodium nitrate	193·7	do.	168·1	do.	148·4	do.	do.
13.	Histidine	189·7	do.	193·2	absent	182·3	do.	poor
14.	Valine	189·6	fair	174·1	fair	150·7	fair	absent
15.	Methionine	188·8	absent	192·6	absent	128·1	absent	do.

16.	*l*-Phenyl alanine	187·9	fair	181·1	do.	147·1	fair	do.
17.	Ammonium tartrate ..	179·7	do.	150·9	poor	182·7	do.	fair
18.	Ammonium oxalate ..	163·4	V. fair	157·1	fair	144·6	do.	do.
19.	Ammonium nitrate	143·3	do.	130·6	poor	100·0	do.	absent
20.	*l*-Leucine	107·1	fair	178·2	absent	141·4	do.	fair
21.	*l*-Aspartic acid	84·0	do.	77·7	do.	43·3	do.	poor
22.	Ammonium sulphate ..	84·0	absent	92·8	do.	69·0	absent	absent
23.	Ammonium dihydrogen phosphate	70·6	fair	75·2	poor	64·5	poor	do.
24.	Ammonium chloride ..	66·4	absent	67·2	absent	73·3	absent	do.
25.	Ammonium bromide ..	63·5	do.	75·6	do.	69·0	do.	do.
26.	Thiourea	38·0	do.	46·8	do.	23·1	do.	do.
27.	Potassium nitrite	0·0	do.	0·0	do.	0·0	do.	do.
28.	Sodium nitrite	0·0	do.	0·0	do.	0·0	do.	do.
29.	Control (no nitrogen) ..	0·0	do.	0·0	do.	0·0	do.	do.

Note.—Poor denotes 1–5 spores; fair 6–10; very fair 11–15 ; and good 16 and more sporse per microscopic field.

Summary of statistical results at 1% level of probability:—

Replication .. non-significant
Treatments .. highly-significant

Fungus	General mean	S.E.	C.D. at 1%
G. musarum ..	147·37	3·19	11·89
G. papayæ ..	137·82	1·75	6·53
C. papayæ ..	116·36	1·53	5·70

General mean ± C.D. at 1% level = moderate growth.

nitrate present in czapek's medium. The following sources of nitrogen were used.

I. *Inorganic compounds.*—Ammonium acetate, ammonium bromide, ammonium chloride, ammonium dihydrogen phosphate, ammonium nitrate, ammonium oxalate, ammonium sulphate, ammonium tartrate, calcium nitrate, magnesium nitrate, potassium nitrate, potassium nitrite, sodium nitrate and sodium nitrite.

II. *Organic compounds.*—Glycine, *d*-alanine, valine, *l*-leucine, *l*-phenyl-alanine, *l*-aspartic acid, glutamic acid, *l*-asparagine, histidine, acetamide, methionine, urea, thiourea and peptone.

Liquid cultures containing 50 c.c. of nutrient solution in 150 c.c. conical flasks were sterilized at 15 lb. pressure for 15 minutes. Previous investigations had indicated that pH 6·0 was best for their growth and sporulation. The same pH was, therefore, adjusted for all media. Pyrex glassware, purest available chemicals and double distilled water were used throughout the investigation. The different media were inoculated with inoculum of equal size (obtained from 15 days old cultures) and they were incubated at 23° C. for 21 days. The dry weights and sporulation were recorded at the end of 1, 2 and 3 weeks. The cultures were filtered on weighed filter-papers and the mycelial mats were thoroughly washed with distilled water so as to remove the salts associated with the mycelium. They were dried to constant weight at 80° C. and weighed rapidly on analytical balance. It was found that in each case the mycelial weight continued to increase with time and there was no difference in general trend of growth in any case. No autolysis of the mycelium was observed within the experimental period. The final weights taken after 21 days have, therefore, been recorded in Table I.

The dry weights recorded in Table I indicate that significantly good growth of all the three fungi was observed on peptone, magnesium nitrate, glycine, ammonium acetate, urea, calcium nitrate, glutamic acid, *d*-alanine, acetamide, *l*-asparagine, potassium nitrate, sodium nitrate, histidine, valine, methionine, *l*-phenyl-alanine, ammonium tartrate and ammonium oxalate. On the contrary, *l*-aspartic acid, ammonium sulphate, ammonium dihydrogen phosphate, ammonium chloride, ammonium bromide and thiourea supported significantly poor growth of all the three fungi. In general all nitrates except ammonium nitrate and all organic compounds except aspartic acid and thiourea supported good growth while ammonium salts of inorganic acids supported significantly poor growth of all the three fungi.

The results of the table also indicate that the source of nitrogen in the medium exerts dominating influence on sporulation. The various compounds supported different degrees of sporulation of the three fungi but in general peptone and all nitrates (except NH_4NO_3), *d*-alanine, urea and acetamide induced good sporulation, whereas all ammonium salts, glycine, glutamic acid, valine, methionine, *l*-phenyl-alanine, *l*-leucine, *l*-aspartic acid and thiourea supported poor to fair sporulation or they completely checked it. There was no growth of all the three fungi on nitrites of sodium and potassium as well as in the complete absence of nitrogen from the medium.

Discussion

In present investigations *Glœosporium musarum*, *Glœsporium papayæ* and *Colletotrichum papayæ* failed to grow on potassium nitrite and sodium nitrite. Brock (1951), Marsden (1954) as well as Tandon and Agarwal (1953), however, found that the fungi investigated by them could utilise nitrites.

Ammonium acetate supported good growth of the three fungi under investigation. Mix (1953), however, found that all the twenty-seven species of Taphrina studied by him were unable to use nitrogen from ammonium acetate. Uppal *et al.* (1938) and Grewal (1955) obtained good growth of *Alternaria burnsii* and *Alternaria tenuis* on ammonium tartrate. Similar results were obtained with the organisms used in present studies. All nitrates (except NH_4NO_3) supported good growth of the three fungi. Fergus (1952) working on *Penicillium digitatum* found that nitrates (except NH_4NO_3) were either not at all utilised or were only poorly utilised.

Tochinai (1926), Leben and Keitt (1948) as well as Wolf (1953) obtained good growth of *Fusarium lini*, *Colletotrichum lini*, *Venturia inæqualis* and *Ustilago zeæ* on glutamic acid. Similar results were obtained for the three fungi used in these investigations. The three fungi gave good growth on histidine, acetamide and methionine but Steinberg (1942) and Brock (1951) reported that they were unsatisfactory for the growth of *Aspergillus niger* and *Morchella esculenta.* Leben and Keitt (1948) and Gordon (1950) found that the growth of *Venturia inæqualis* and *Endoconidiophora moniliformis* was good on urea which was also found to be good source for the present three fungi. It, however, supported poor growth of the fungi investigated by Uppal *et al.* (1938) and Srivastava (1951).

Peptone and all nitrates (except NH_4NO_3) induced good sporulation of the three fungi used in the present study. Patel *et al.* (1950), Timnick *et al.* (1951) and Grewal (1955) also found potassium nitrate to be a good

source of nitrogen for the sporulation of *Pestalotia psidii*, *Melanconium fuligineum*, and *Alternaria tenuis* respectively. The three fungi used in present investigation failed to sporulate on ammonium bromide, ammonium chloride and ammonium sulphate. Grewal (1955) obtained similar results with the above compounds. Patel *et al.* (1950), Mathur *et al.* (1950) and Timnick *et al.* (1951) reported that ammonium sulphate was a poor source of nitrogen for sporulation of fungi investigated by them.

G. papayæ failed to sporulate on glycine, *l*-leucine and glutamic acid but they supported fair sporulation of *G. musarum* and *C. papayæ*. Steinberg (1942) obtained good sporulation of *Aspergillus niger* on above compounds. Peptone supported good sporulation of all the three fungi. Mathur *et al.* (1950), Marsden (1954) and Grewal (1955) also found it to be good for the sporulation of *Colletotrichum lindemuthianum*, *Hormodendrum resinæ* and *Alternaria tenuis*.

The present study indicates that all nitrates (except NH_4NO_3) and a number of complex nitrogen compounds supported good sporulation while a majority of ammonium salts as well as some other substances suppressed sporulation of the three fungi. The setæ of *C. papayæ* which were the basis of the separation of Colletotrichum from Glœosporium were also influenced by nutrition. The number as well as the presence or absence of setæ of *G. papayæ* was controlled by the nature of the nitrogen compound added to the basal medium.

SUMMARY

Effect of twenty-eight different nitrogen compounds on dry weight and sporulation of *Glœosporium musarum* isolated from bananas, *Glœosporium papayæ* and *Colletotrichum papayæ* isolated from papayas, was studied in liquid cultures. There was no growth of the three fungi on nitrites of sodium and potassium as well as in complete absence of nitrogen from the medium. All nitrates (except NH_4NO_3) and majority of organic compounds supported good growth while ammonium salts of inorganic acids and thiourea supported significantly poor growth of all the three fungi.

Results also indicate that the source of nitrogen in the medium exerts dominating influence on the sporulation. *d*-alanine, urea, acetamide and all nitrates (except NH_4NO_3) induced good sporulation. Most of the ammonium salts, glycine, glutamic acid, valine, methionine, *l*-phenyl-alanine, *l*-leucine, *l*-aspartic acid and thiourea supported poor to fair sporulation or completely checked it. The number as well as the presence or absence of setæ of *C. papayæ* was controlled by the nature of the nitrogen added to the basal medium.

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